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THE BASE PROMOTED OLIGOMERIZATION OF 15-DEHYDRO-PROSTAGLANDIN B_1: DIMER FORMATION AND STRUCTURAL IMPLICATIONS FOR A COMPLEX MIXTURE TERMED PGBx

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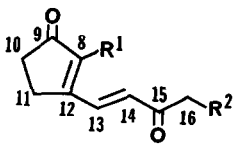
Summary: The base promoted oligomerization of 15-dehydro-PGB_1 (1a) results in the formation of six dimers via a Michael addition pathway. The simultaneous operation of multiple reaction sites as required by dimer formation has major consequences for the structural complexity of the oligomeric mixture PGBx.

Recently we reported the isolation and characterization of six dimers from the oligomeric mixture formed upon treatment of the 15-dehydro-PGB_1 analog 1c with dilute ethanolic KOH. The oligomerization pathway leading to dimer formation, which proceeded via Michael addition through multiple reaction sites, provided for the first time structural insights into the complex mixture termed PGBx. The term PGBx currently refers to a complex mixture derived from treatment of 15-dehydro-PGB_1 methyl ester (1b) with 1 N ethanolic KOH for 4 hours at 80°.[2] PGBx has been reported to protect against the loss of oxidative phosphorylation *in vitro* in isolated mitochondria[3a] and reverse *in vivo* damage in animals subjected to episodes of myocardial[3b] and cerebral[3c] ischemia. Recently Toda et. al.[4] reported the formation of two dimers from the prolonged base treatment of PGB_1, an earlier precursor of a complex mixture also termed PGBx,[2] that are markedly different in structure from the six dimers derived from the 15-dehydro-PGB_1 analog 1c. Two dimers have also been reported by Polis et. al.[5] from the treatment of 15-dehydro-PGB_1 methyl ester (1b) with dilute ethanolic KOH. We wish to report here that, contrary to the report of Polis, treatment of 15-dehydro-PGB_1 as either the free acid (1a) or methyl ester (1b) with dilute ethanolic KOH results in the formation of six dimers. The oligomerization pathway indicated by presence of six dimers indicates a PGBx mixture many magnitudes more complex both structurally and stereochemically than one indicated by the report of only two dimers.



1a, $R^1 = (CH_2)_6CO_2H$, $R^2 = (CH_2)_3CH_3$
b, $R^1 = (CH_2)_6CO_2CH_3$, $R^2 = (CH_2)_3CH_3$
c, $R^1 = CH_2CH_3$, $R^2 = CH_3$

Treatment of 1a with 0.05 M ethanolic KOH (8.3 mg/ml) for 90 minutes at 22° gave a crude reaction product from which the dimer component was separated by chromatography on Sephadex LH-20 (CH_3OH).[6] After treatment with diazomethane, the dimer fraction was separated by HPLC on

two 10 mm x 25 cm LiChrosorb columns in series (35-45% EtOAc/C_6H_{12}) into six components.[7] A molecular formula of $C_{42}H_{64}O_8$, i.e. $(C_{21}H_{32}O_4)_2$, was determined for each component by HRMS measurement of the molecular ion.[8]

Two distinctly different dimer types were indicated by the spectral data. Dimers 2b-5b exhibited UVmax at 296 and 238 nm and conjugated C=C IR absorptions at 1585 and 1640 cm^{-1} whereas dimers 6b and 7b had a single UVmax at 238 nm and a conjugated C=C IR absorption at 1640 cm^{-1}.[9] Dimers 2b-5b exhibited a strong fragment ion at m/e 348, i.e. $C_{21}H_{32}O_2$, while only a weak m/e 348 fragment ion was present in dimers 6b and 7b.[8] The structural assignments of the individual dimers follow from the previously established structures of the dimers derived from the analog 1c and are given in Figure 1.[10] This correspondence is evident from a comparison of ^{13}C and ^1H chemical shifts of dimers derived from 1a and 1c listed in Tables 1 and 2.[10]

Dimers 2a-7a are derived from 1a via Michael addition in which two nucleophilic (C-10 and C-16) and two acceptor (C-13 and C-14) sites are active as indicated in Figure 1. Dimers 2a and 3a, a diastereomeric pair, are formed by the addition of the C-10 enolate of 1a to C-14' of a second unit of 1a. Dimers 4a and 5a, a second diastereomeric pair, arise from the addition of the C-10 enolate of 1a to C-13' of a second unit. Dimer 6a arises from the initial addition of the C-16 enolate of 1a to C-13' of a second unit leading to a new enolate which internally cyclizes by addition of C-14' to C-14 resulting in the formation of a cyclopentanone ring with linkages at 16-13' and 14-14'. In a similar manner, dimer 7a results from the initial addition of the C-16 enolate to C-14' followed by the internal cyclization by addition of C-13' to C-14 giving rise to a cyclopentanone ring with 16-14' and 14-13' linkages. The single addition dimers 2a-5a retain a residual 13,14-unsaturation while dimers 6a and 7a, resulting from double addition, lack a residual 13,14-unsaturation.

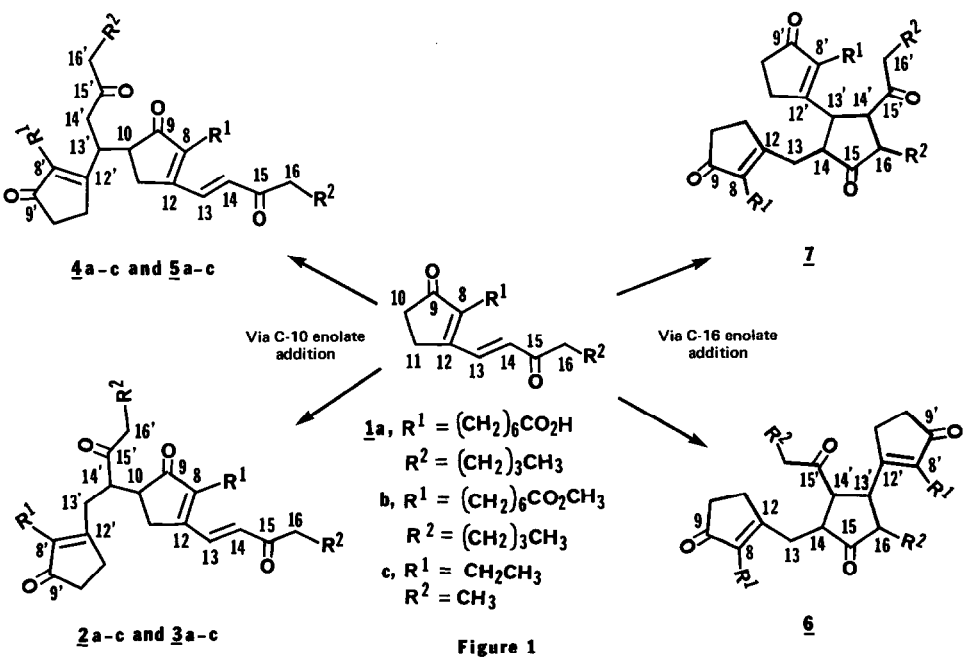


Figure 1

Table 1. A Comparison of ^{13}C NMR Chemical Shifts[a] of Dimers 2-7 derived from 15-Dehydro-PGB$_1$ (1a) and the Analog 1c.[10]

C #	2b	2c	3b	3c	4b	4c	5b	5c	6b	6c	7b	7c
C-8	147.6^s	148.9^s	147.1^s	148.4^s	147.6^s	148.9^s	147.4^s	148.7^s	142.2^s	143.5^s	142.0^s	143.6^s
C-8'	142.5^s	143.7^s	142.9^s	144.1^s	142.5^s	143.8^s	142.0^s	143.4^s	144.7^s	146.6^s	145.0^s	146.6^s
C-9	208.8^s	207.5^s	207.5^s	208.2^s	208.4^s	208.3^s	207.7^s	208.0^s	208.6^s	208.8^s	209.0^s	208.5^s
C-9'	208.9^s	208.9^s	208.7^s	208.7^s	208.8^s	208.8^s	208.7^s	208.4^s	207.9^s	208.0^s	208.1^s	207.8^s
C-10	45.9^d	46.1^d	46.6^d	46.6^d	46.1^d	46.1^d	45.3^d	45.6^d	34.3^t	34.4^t	33.9^t	34.2^t
C-12	159.6^s	159.0^s	159.3^s	158.8^s	158.8^s	158.2^s	157.9^s	157.4^s	168.8^s	165.6^s	168.8^s	165.6^s
C-12'	168.8^s	168.3^s	168.2^s	167.7^s	170.9^s	170.5^s	171.9^s	171.2^s	166.8^s	168.3^s	166.2^s	167.7^s
C-13	133.2^d	133.1^d	133.4^d	133.2^d	133.3^s	133.1^d	133.3^s	133.2^d	31.0^t	31.0^t	31.2^t	30.4^t
C-13'					37.1^d	37.2^d	37.2^d	37.6^d	48.2^d	48.2^d	46.9^d	46.2^d
C-14	131.3^d	131.0^d	131.2^d	130.9^d	131.2^d	130.9^d	130.8^d	130.6^d	56.2^d	56.2^d	56.3^d	57.5^d
C-14'	49.8^d	49.6^d	49.1^d	49.0^d					50.4^d	50.4^d	51.3^d	50.7^d
C-15	199.6^s	200.0^s	199.7^s	200.0^s	199.5^s	200.0^s	199.8^s	200.0^s	214.6^s	214.7^s	214.7^s	214.5^s
C-15'	210.7^s	211.2^s	210.7^s	211.1^s	208.8^s	208.8^s	209.5^s	209.1^s	209.0^s	209.3^s	209.7^s	209.6^s
C-16									49.0^d	49.0^d	52.1^d	47.7^d

a) The chemical shifts (δ, $CDCl_3$) of carbons directly involved in dimer bond formation are underlined.

Table 2. A Comparison of ^1H NMR Chemical Shifts[a] of Dimers 2-7 derived from 15-dehydro-PGB$_1$ (1a) and the Analog 1c.[10]

H #	2b	2c	3b	3c	4b	4c	5b	5c	6b	6c	7b	7c
H-10	2.82	2.86	2.83	2.88	2.76	2.78	2.57	2.60				
H-13'					3.77	3.70	3.52	3.52	3.20	2.04	3.29	3.35
H-14'	3.43	3.42	3.50	3.52					2.86	2.91	3.04	3.00
H-14									3.12	3.20	2.58	2.66
H-16									2.40	2.40	2.77	2.72

a) The chemical shifts (δ, $CDCl_3$) of protons attached to carbons directly involved in dimer bond formation are included in Table 2.

Oligomeric mixtures derived from a reaction pathway utilizing the multiple reaction sites involved in the formation of six dimers would be many magnitudes more complex than mixtures anticipated from a pathway based on only two dimers. The complexity of oligomeric mixtures in which the next higher oligomer arises from either C-10 or C-16 enolate addition of 1a to a residual 13,14-unsaturation[11] rapidly increases with each additional unit of 1a added[12] and can be illustrated in trimer formation. Addition of the C-10 enolate of 1a to either C-13' or C-14' of the diastereomeric dimer pairs 2a-3a and 4a-5a leads to 4 structurally isomeric trimers with 4 chiral centers each.[13] Each of the trimers formed in this manner retain a residual 13,14-unsaturation necessary for conversion to tetramers. In contrast, C-16 enolate addition to either C-13' or C-14' of the diastereomeric dimer pairs 2a-3a and 4a-5a by the double addition pathway results in 4 additional structurally isomeric trimers with a new cyclopentanone ring and 6 chiral centers.[13] Although such trimers contribute greatly to the overall complexity of the oligomeric mixture, a residual 13,14-unsaturation required for conversion to tetramers is lacking. It is on the basis of such complexity[12] that we believe the direct structural elucidation of individual components of PGBx, estimated to be in the hexamer-octamer range,[2] does not represent a feasible approach.

We will report in the near future on more detailed structural characteristics of trimers and higher oligomers derived from 1a and related analogs. The biological activity of oligomers derived from 1a will be reported separately.

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References and Notes

1. G. L. Nelson and G. L. Verdine, Tetrahedron Lett., 1967 (1982).
2. a) B. D. Polis, S. Kwong, E. Polis and G. L. Nelson, Physiol. Chem. Phys., 12, 167 (1980). b) B. D. Polis, S. Kwong, E. Polis, G. L. Nelson and H. W. Shmukler, Physiol. Chem. Phys., 11, 109 (1979). In the past, the term PGBx has been applied to complex mixtures derived by very vigorous KOH treatment of PGB_1, cis-PGB_1, 13,14-dehydro-PGB_1, PGA_1 and PGE_1; cf. ref. 2a.
3. a) B. D. Polis, E. Polis and S. Kwong, Proc. Natl. Acad. Sci. USA, 76, 1598 (1979). b) E. T. Angelokas, R. I. Riley and B. D. Polis, Physiol. Chem. Phys., 12, 81 (1980). c) G. Moss, T. Magliocchetti and R. Quarmby, Surg. Forum, 29, 513 (1978). See also ref. 1 for further details.
4. M. Toda, S. Takaoka, M. Konno, S. Okuyama, M. Hayashi, N. Hamanaka and T. Iwashita, Tetrahedron Lett., 1477 (1982).
5. B. D. Polis, E. Polis and S. Kwong, Physiol. Chem. Phys., 13, 531 (1981).
6. Oligomerization of 15-dehydro-PGB_1 (1a) as the free acid was preferable to the methyl ester 1b since transesterification of 1b resulted in complications in the HPLC isolation of individual dimers. A reaction period of 90 minutes gave 56% unreacted monomer, 23% dimer and 21% higher oligomer that was principally trimer.
7. The overall percentage of dimers 2a-5a decreases relative to dimers 6a and 7a with increasing reaction time. The distribution of dimers 2a-5a (ca. 25% 2a, 8% 3a, 33% 4a and 34% 5a) and dimers 6a and 7a (ca. 50:50) remains essentially constant with increasing reaction time. The dimers 2a-7a correspond structurally to the six dimers derived from the analog 1c.[1]
8. High resolution mass spectrometry (HRMS) measurements of dimers 2b-7b were performed by Dr. D. T. Terwilliger and M. Davis of the Mass Spectrometry Laboratory, the University of Pennsylvania, Philadelphia, PA. We gratefully acknowledge their able assistance.
9. 15-Dehydro-PGB_1 methyl ester (1b) exhibits a UVmax at 296 nm and a conjugated C=C IR absorption at 1585 cm^{-1} while 13,14-dihydro-15-dehydro-PGB_1 methyl ester has a UVmax at 238 nm and a conjugated C=C absorption in the IR at 1640 cm^{-1}.
10. The structures of 5 of the 6 dimers derived from the analog 1c have been independently confirmed and the stereochemistry established by X-ray crystallographic determinations carried out by Dr. G. T. DeTitta, the Medical Foundation of Buffalo, Buffalo, N.Y. Personal communication. Details will be reported elsewhere.
The ^{13}C NMR spectra of dimers 2b-7b were determined at 62.9 MHz using a Bruker WM-250 NMR instrument. We gratefully acknowledge the assistance of Dr. G. Furst of the Department of Chemistry, the University of Pennsylvania, Philadelphia, PA.
The ^{1}H NMR spectra were determined at 360 MHZ using a Bruker WH-360 NMR instrument at the Middle Atlantic NMR Facility, the University of Pennsylvania, Philadelphia, PA supported by Grant NIH-RR-542. We gratefully acknowledge the assistance and suggestions of Drs. G. McDonald and D. Huang. The assignment of overlapping resonances was facilitated by selective ^{1}H-^{1}H decoupling experiments.
11. The following evidence supports C-10 and C-16 enolate addition to a residual 13,14-unsaturation as the primary pathway for chain growth. The single addition dimers 2a-5a, which retain a residual 13,14-unsaturation, are converted into primarily tetramers and hexamers while the double addition dimers 6a and 7a are recovered unchanged when resubjected to the original reaction conditions. The reaction of monomer 1a in the presence of excess dimers 2a-5a results in the formation of trimer at a considerably faster rate than tetramer formation. Trimers resulting from both C-10 and C-16 enolate addition have been isolated. See also ref. 13.
12. Considering only oligomer chain formation by C-10 enolate addition to either the C-13 or C-14 acceptor sites coupled with the creation of two new chiral centers for each unit added, 1a could lead to 4 pairs (2×2^2) of enantiomeric dimers, the dimers to 32 pairs (4×2^4) enantiomeric trimers, the trimers to 256 pairs (8×2^6) of enantiomeric tetramers, the tetramers to 2048 pairs (16×2^8) of enantiomeric pentamers, the pentamers to 16,384 (32×2^{10}) pairs of enantiomeric hexamers, etc. The degree of complexity arising from the formation of new chiral centers was previously underestimated.[1]
13. Trimers derived from C-10 enolate addition to dimers 2a-5a are separable by HPLC from trimers derived from C-16 enolate addition via the double addition pathway. Details of the structure and activity of such trimers will be reported in the near future.

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